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THE NECESSITY OF CARBON DIOXID FOR THE GROWTH OF B. TUBERCULOSIS

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Last year one of us noted that if the respiratory CO_2, evolved by a freshly planted virulent culture of the tubercle bacillus, was removed, growth did not take place. The bacilli remained in a nonreproductive state but viable, as shown by guinea-pig inoculation two weeks later.

The culture was isolated, in February 1917, by Dr. John Hermanies, from postmortem pulmonary lesions in man, by guinea-pig inoculation and cultivation on rabbit blood agar. Repeated tests made shortly after isolation and off and on up to the present time have confirmed the original observation regarding the necessity of CO_2. In the earlier tests the respiratory CO_2 was absorbed by joining the culture tube to another test tube, containing from 5-10 c c of saturated barium hydroxid solution, by means of rubber tubing. As controls, similar cultures were attached to tubes of distilled water. As the pellicle of barium hydroxid formed, it was shaken down daily. When this shaking down of the pellicle was performed faithfully, the growth was completely inhibited. But when the pellicle was allowed to stand, as it was usually in the later stages of the observations, the absorption was interfered with, CO_2 accumulated, and growth then usually began as isolated islands. This differed markedly from the growth in the water tubes which started all over the inoculated surface and soon yielded a confluent nodular layer. In our later experiments we have used sodium hydroxid as this requires no attention. Figure 1 shows the results of growth against sodium hydroxid and water, respectively.

Most of these experiments have been performed on nutrient agar made up from the extract of lean beef with 1% Witte's peptone, 0.5% Na_2HPO_4 and 6% glycerin. This was neutralized with normal sodium hydroxid to a reaction of ∓ 1.5 — ∓ 2.0 phenolphthalein. It was tubed in 5 c c quantities and autoclaved. To each tube was then added 0.5 c c defibrinated rabbit blood. On this medium, in the tubes attached to water, the growth was luxuriant and confluent in 2 to 3 weeks at

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37 C. After inoculation there was always, however, a period of "lag" during which no proliferation could be noted.

We are now engaged in a closer analysis of optimum CO_2 pressure which will also be supplemented by a study of the oxygen requirements. Reference to the accompanying table will show that one might be led to the belief that "lag" is a latent period during which CO_2 accumulates to an optimum concentration. Accompanying this increase in CO_2 pressure is a gradually diminished O pressure. When both O and CO_2 pressures reach an optimal point, growth starts. For example, as shown in the table, Tube III under an initial pressure of 17 mm. of CO_2 on December 31, showed approximately the same growth as Tube

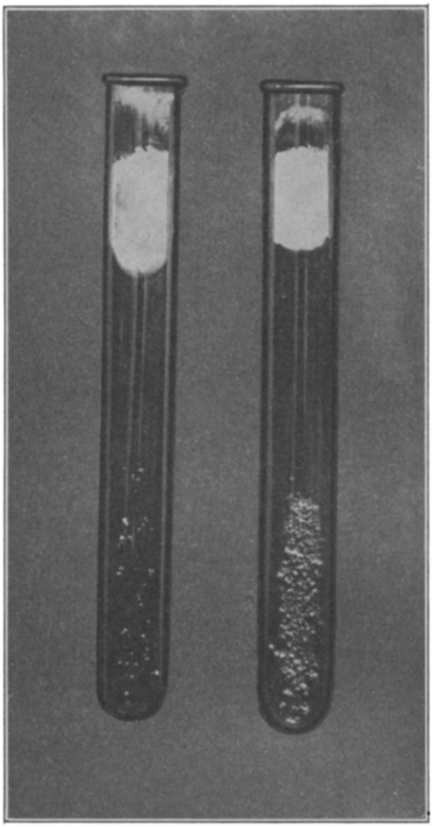

Fig. 1.—Showing the effect of attaching cultures to sodium hydroxid solution and water, respectively.

VI did on December 26, which was under an initial pressure of 47 mm. It should be noted that the growths in this series all occurred with certain limits of CO_2 pressure; e. g., Tubes III-VI. In Tubes IV and V, it will be noted that the CO_2 escaped and, while growth commenced, it soon ceased. In Tube I, which was under atmospheric (laboratory, 7 mm.) CO_2 pressure and in Tube VII in which the CO_2 pressure was 77 mm., there was no growth. We have realized the necessity of investigating the need for oxygen. All the recorded observations we have seen emphasize the necessity of a supply of free oxygen for the growth of B. tuberculosis on artificial mediums. Just how much is needed has not been determined apparently. However, the experiments of Adams[1] show that concentrations above the atmospheric inhibit growth on artificial mediums. Now, in a closed system such as has been described above, not only does CO_2 accumulate but the oxygen supply rapidly diminishes. However, there is usually enough oxygen present to yield a confluent layer of growth.

COMPARATIVE TESTS WHICH ILLUSTRATE THE EFFECT OF DIFFERENT CO_2 PRESSURES ON THE GROWTH OF B. TUBERCULOSIS

Dates of Observation	December 18	December 26		December 31		January 3		January 8		
Barometer = 750 mm. Temperature = 20 C.	CO_2 in mm.	Saprophytic	Nonsaprophytic	Saprophytic	Nonsaprophytic	Saprophytic	Nonsaprophytic	Saprophytic	Nonsaprophytic	CO_2 %
I	H_2O 7.7△	+	0	+	0	++	0	+++	+	6.3
II	NaOH 0	+	0	+	0	+	0	+	0	0.0
III	17	++	+	+++	+	+++ +++	++	+++ +++	++	8.25
IV	27	++	++	++	++	++	++	+++	++	2.0*
V	37	+	++	++	++	++	++	++	++	1.2*
VI	47	+++	+	+++	+	+ +++	++	++ +++	++	6.8
VII	77	0	0	0	0	0	0	0	0	13.0

△ Room pressure = 7.7 mm.
+ sign indicates the degree of growth.
+++ means a luxuriant confluent nodular layer of growth.
* evidently CO_2 escaped.

In the accompanying table, we refer to saprophytic and nonsaprophytic strains. The nonsaprophytic is the one with which most of our experiments were performed. The saprophytic is called such because it grows a little more luxuriantly on all mediums than the parent strain from which it was derived. It originated in the following interesting manner:

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[1] Biochem. Jour., 1912, 6, p. 297.

A freshly inoculated transplant, on the glycerin rabbit's blood agar previously described, was attached to a tube of barium hydroxid, Oct. 13, 1917, and kept in the incubator at 35-37 C. until Dec. 10, 1917. It had not been disturbed for at least a month. The hydroxid tube was covered with a thick scum of the carbonate and the culture showed 9 islands of growth from 2-4 mm. in diameter. Four of the layer islands, which were of a greenish color, showed dryish wrinkled salmon-colored outgrowths from the edges—thus producing areas of growth from 10-12 mm. in diameter. This outgrowth was composed of completely acid-fast rods resembling the tubercle bacillus and not differing in morphology markedly from the bacilli in the central raised portions of the islands. Thinking that this might be a variant it was subcultured to mediums of the same composition but neutralized with sodium carbonate. In 6 days it yielded a growth as luxuriant as the original culture from which it was derived would yield in 3-4 weeks. Transplants from the central portion of the above areas grew extremely slowly on this medium. This saprophytic variant has shown another peculiarity in that its grows luxuriantly at partial tension, namely, when joined to a culture of B. subtilis, whereas experiments like this performed on several occasions previously with the parent strain always yielded negative results.